Monograph

No 19

Respiratory Allergy

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RESPIRATORY ALLERGY

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RESPIRATORY ALLERGY

CONTENTS

SUMMARY

Various chemicals and proteins of industrial importance are known to cause respiratory allergy, with occupational asthma being the most important manifestation of respiratory allergy for the chemical industry. This monograph describes the clinical syndromes, the mechanisms associated with occupational respiratory hypersensitivity, the clinical criteria used for diagnosis and methods available currently for the prospective identification of potential respiratory allergens.

As yet there exist no fully validated or widely applied predictive methods or internationally harmonised guidelines for the prospective identification of potential respiratory allergens.

Certain classes of chemicals are commonly associated with occupational respiratory allergy. There is insufficient information to predict respiratory sensitisation potential from analysis of structure alone. Some physicochemical characteristics and biological properties appear important correlates of respiratory sensitisation. Reactivity with proteins is likely to be relevant. Effective use of structure-activity relationships for the prospective identification of respiratory sensitisers will not become a reality until more is known about the mechanism of sensitisation and the reactivity of different chemical classes. Since no suitable *in vitro* methods are currently available, most attention is given to animal models for their detection.

The most promising predictive animal methods are the mouse IgE test and guinea pig models. Work in mice has focused upon events occurring during the induction phase of sensitisation following primary encounter with the test chemical. In contrast, guinea pig models have been used primarily to identify respiratory allergens as a function of elicitation reactions induced in previously sensitised animals. The mouse IgE test is suitable for the evaluation of chemicals whereas the guinea pig models appear to be suitable both for the evaluation of chemicals and proteins.

Given the possible serious health effects of respiratory allergy, an early identification of respiratory sensitisers is important. The two methods described in this monograph require to be developed further and the production of a detailed protocol for these methods will facilitate further validation. Selection of substances for the validation process should be based on occupational health data and epidemiological studies. Together, this information will allow for the two types of risk assessment associated with respiratory allergy: the risk that exposure to a material will induce sensitisation in an individual and elicit allergic reactions in a previously sensitised individual.

SECTION 1. INTRODUCTION

Respiratory sensitisation is an immune state (frequently but not exclusively, the presence of specific IgE antibodies) which is likely to result in symptoms of respiratory disease or distress when a particular allergen is inhaled. Respiratory allergy is the clinical manifestation of this state, with bronchial asthma and/or rhinitis (e.g. hay-fever) constituting the most important disease.

For centuries exposure to various substances and chemicals in the workplace has resulted in respiratory sensitisation and allergy. Baker's allergy, for instance, is a well-known example of occupational respiratory disease, in which sensitised individuals exhibit symptoms of bronchial asthma or rhinitis following exposure to different kinds of flour (Musk *et al,* 1989). The number of known allergens is increasing and includes both proteins and low molecular weight chemicals (Cullen *et al*, 1990).

The immunologic reactions underlying the symptoms associated with respiratory allergy in sensitised individuals are only partially characterised. In most cases the symptoms can be successfully treated. Much less is known about the mechanisms which result in respiratory sensitisation. While individuals already sensitised may exhibit symptoms following inhalation of extremely low concentrations of a respiratory allergen, little is known about the doses necessary for inducing sensitisation, even with common allergens like pollens, house dust mite or animal dander. Relatively high concentrations of an allergen, possibly in just one or a few exposures, are believed to be important in the induction stage. Little evidence supports the common view that repeated exposure to low doses will eventually lead to sensitisation (Karol and Thorne, 1988). The available evidence indicates that a variety of factors, both genetically determined and acquired, will influence predisposition to respiratory allergy.

One of the most important issues in occupational allergy, therefore, is determining the critical exposure conditions which result in sensitisation and those which will cause respiratory symptoms in previously sensitised individuals. Armed with such information industries involved in the production and handling of compounds with allergenic potential can take measures to prevent exposure of workers to levels which might otherwise lead to respiratory sensitisation and allergy.

In order to review what is presently known in the area of respiratory sensitisation and allergy and to investigate future needs, a task force was established with the following aims:

- review the syndromes and immunological mechanisms underlying respiratory sensitisation and allergy;
- review animal and *in vitro* models currently used for predicting the potential of a substance to cause respiratory allergy and assess their present state of validation;
- determine the immunological and/or clinical criteria necessary to establish that a substance has induced respiratory sensitisation in people;
- recommend approaches to risk assessment in respiratory sensitisation and allergy.

The definitions of the specific terms used in this Monograph are given in Appendix A.

SECTION 2. BACKGROUND

Allergic respiratory disease can take several forms.

Hypersensitivity pneumonitis, or extrinsic allergic alveolitis, is well known in occupational medicine. Many forms are known under names related to either the occupation or the responsible substance: i.e. farmers' lung, mushroom workers' lung, malt workers' lung, bird fanciers' disease, bagassosis, suberosis, byssinosis etc. Most of them are relatively well studied because of the possible serious long-term sequelae. Nearly all are due to organic dust, i.e. material from animal, vegetable or bacterial origin. For most of the chemical industry, hypersensitivity pneumonitis is rarely a cause of occupational disease. Allergic bronchopulmonary aspergillosis is another form of allergic respiratory disease. It is a condition caused by *Aspergillus* spores and is characterised by pulmonary eosinophilia (Slavin, 1983) and is rarely an occupational disease. These subjects are not discussed further in this monograph.

Another form of respiratory disease is associated with asthma and/or rhinitis. This form of respiratory allergy can be caused by both high-molecular weight substances, usually proteins, and by low molecular weight chemicals.

A list of agents causing asthma in selected occupations is given in Table 1. Occupational asthma caused by proteins is associated primarily with exposure to animal allergens and handling of protein powders. An example of the latter was asthma among workers in the detergent industry in the late sixties and early seventies, caused by exposure to dusty proteolytic enzyme powders. The occurrence of occupational allergy was controlled successfully by reducing the level of enzyme in the factory environment, with the introduction of non-dusty enzyme products and the implementation of appropriate containment and ventilation (Gilson *et al*, 1976). Studies by Pepys *et al* (1985) and Zetterstrom (1977) confirmed that the widespread use of non-dusty enzymes in detergents has not caused respiratory allergy in consumers.

The best documented cases of occupational rhinitis are due to exposure to animal allergens. There are few published data on occupational rhinitis resulting from exposure to low molecular weight chemicals. Occupational rhinitis is less well studied because of the lack of severity in the clinical symptoms compared with asthma and the difficulty in establishing the aetiology.

Occupational asthma resulting from exposure to low molecular weight chemicals is of continued concern to the chemical industry. The best examples of chemicals which have the potential to sensitise include some isocyanates, reactive dyes and acid anhydrides. For some applications of isocyanates, the problem has largely been solved by the development of "capped" isocyanates and

TABLE 1 TYPICAL EXAMPLES FOR AGENTS CAUSING ASTHMA IN SELECTED OCCUPATIONS (Sheffer, 1991a)

Occupation or Occupational Field	Agent
laboratory animal workers, veterinarians	dander and urine proteins
food processing	shellfish, egg proteins, pancreatic enzymes, papain, amylase
dairy farmers	storage mites
poultry farmers	poultry mites, droppings and feathers
granary workers	storage mites, aspergillus, indoor ragweed, and grass pollen
research workers	locusts
fish food manufacturing	midges
detergent manufacturing	*Bacillus subtilis* enzymes
silk workers	silk-worm moths and larvae
	Plant Proteins:
bakers	flour
food processing	coffee bean dust, meat tenderizer (papain), tea
farmers	soy bean dust
shipping workers	grain dust (molds, insects, grain)
laxative manufacturing	ispaghula
sawmill workers, carpenters	wood dust (western red cedar, oak, mahogany, zebrawood, redwood, Lebanon cedar, African maple, eastern white cedar)
electric soldering	colophony (pine resin)
cotton textile workers	cotton dust
nurses	psyllium
	Inorganic Chemicals:
refining	platinum salts
plating	nickel salts
diamond polishing	cobalt salts
stainless steel welding	chromium salts
manufacturing	aluminum fluoride
beauty shop	persulphate
refinery workers	vanadium
welding	stainless steel fumes
	Organic Chemicals:
manufacturing	antibiotics, piperazine, methyl dopa, salbutanol, cimetidine
hospital workers	disinfectants (sulphathiazole, chloramine, formaldehyde, psyllium, glutaraldehyde)
anaesthesiology	enflurane
poultry workers	aprolium
fur dyeing	paraphenylene diamine
rubber processing	formaldehyde, ethylene diamine, phthalic anhydride
plastics industry	toluene diisocyanate, hexamethyl diisocyanate, diphenylmethyl isocyanate, phthalic anhydride, triethylene tetramines, trimellitic anhydride, hexamethyl tetramine
automobile painting	dimethyl ethanolamine toluene diisocyanate
foundry worker	furfuryl alcohol resin

isocyanates with very low vapour pressures. All published cases of respiratory allergy to low molecular weight chemicals are derived from occupational rather than consumer exposure.

Nevertheless, occupational asthma has received little attention in the past, to the extent that even the incidence and severity of the problem is not well established. Several factors may have contributed to this lack of attention:

- Clinical attention was focused more on the much more prevalent pneumoconioses (with and without tuberculosis), and the above-mentioned extrinsic allergic alveolitis, because of their serious long-term consequences.

- Individual cases are easily lost in the large pool of "intrinsic" and "atopic" asthma patients; thereby, the diagnosis is often delayed, and the relationship to exposure to a specific substance can easily be missed.

- There are only a few epidemiological studies; in fact, with a few exceptions, most "sensitising" chemicals are classified as such on the basis of a limited number of clinical case-studies, most of them without relevant exposure data. Levels and route of exposure required to induce respiratory sensitisation, are largely unknown.

- It is not always easy to merge clinical and epidemiological data. While signs and symptoms in individuals, allergic to a given substance, may indicate a strongly allergenic substance, the percentage of exposed people that develop allergy may be quite low. To complicate matters further, not all sensitised individuals (e.g. with elevated specific IgE) develop allergic disease.

- Allergy, with its high species and individual specificity, was until recently regarded more as an aberration of the individual than as a hazard of the relevant chemical. Even in quite recent textbooks on toxicology, allergic asthma is not even mentioned under the toxic responses of the respiratory system, and gets only passing attention in the discussion of the immune system.

- Compared with contact hypersensitivity, the other major form of occupational allergy associated with exposure to chemicals, no fully validated models are available to identify the hazard of respiratory sensitisation and allergy.

With emerging evidence for the ability of some chemicals to cause respiratory allergy in man, there is now a need to develop methods which will permit their prospective identification. Such candidate methods should be validated with chemicals of known and different allergenic potential.

SECTION 3. DEFINITION OF RESPIRATORY SENSITISATION AND ALLERGY

Respiratory sensitisation is an immune status whereas respiratory allergy is the clinical disease. Respiratory sensitisation results from an immune response to antigen (frequently, but not exclusively, inhaled exogenous antigen) which may result in clinical hypersensitivity upon subsequent exposure to the same or similar antigen. An allergic response characteristically requires at least two encounters with the same antigen. As a result of the first exposure the individual mounts a primary immune response which results in sensitisation (the induction or sensitisation phase). If the sensitised individual comes into contact with the same antigen subsequently a clinical allergic reaction may be provoked (the elicitation phase). Allergic reactions may either be attributable to antibody or cell-mediated immune responses. Allergic reactions in the respiratory tract induced by exposure to exogenous antigens are almost invariably associated with specific antibody responses, frequently, but not exclusively, of the IgE class.

Asthma can be defined as a lung disease characterised by

- airways obstruction which in most patients is reversible, either spontaneously or after treatment,
- airways inflammation and
- increased airway responsiveness to a variety of stimuli (Sheffer, 1991a).

Occupational asthma is defined as a respiratory disease characterised by variable bronchial obstruction and variable bronchial hyperreactivity caused by specific agents inhaled at work (Maestrelli, 1992). Rhinitis is a disease that involves inflammation of the nasal mucous membrane which is characterised by periods of nasal discharge, sneezing, and congestion that persist for an average of at least ½ to 1 hour per day (Mygind and Weeke, 1983).

Pseudo-allergic reactions (e.g. patients exposed to X-ray contrast media) which are often clinically indistinguishable from true allergic reactions are outside the scope of this Monograph.

SECTION 4. MECHANISMS UNDERLYING RESPIRATORY SENSITISATION AND ALLERGY

4.1 INTRODUCTION

Allergic reactions in the respiratory tract resulting from exposure to exogenous antigens are usually, but not exclusively effected by specific antibody. For ease two major classes of pulmonary allergic reactions to external antigens can be distinguished:

- **Hypersensitivity pneumonitis** results from inflammatory reactions caused by short- or long-term intermittent exposure to certain protein antigens. Examples include Farmer's Lung caused by inhalation of *Micropolyspora faeni* antigens and Cheese Worker's Lung caused by *Penicillium roqueforti* antigens. The disease is associated characteristically with the presence of antigen-precipitating IgG antibody. Pathogenesis may, however, also involve the activation of complement and cell-mediated immunity.

- **Allergic asthma and rhinitis** are most commonly immediate-onset reactions (within 1 hour and often within minutes of exposure) and result from the local release of inflammatory mediators. Such reactions are usually, although not exclusively, effected by IgE antibody. Asthmatic reactions may also be persistent or have a late-onset and it is possible here that other types of immune reaction play a part.

For a more detailed account of pulmonary allergic reactions the reader is referred to various texts (Turner-Warwick, 1978; Fireman and Slavin, 1990; Thomson *et al*, 1990).

It is allergic asthma that constitutes the most common form of respiratory allergy to low molecular weight chemicals.

4.2 CELLULAR AND MOLECULAR MECHANISMS

The most important event during the induction phase of respiratory sensitivity is the generation of an antibody response, and in particular production of antibodies which are able to bind to tissue mast cells. In the past such antibodies have been designated cytotropic. It was recognised that two types of cytotropic antibody could be distinguished: homocytotropic and heterocytotropic. As these names imply, the former were found to bind only to mast cells of the same, or similar, species, while the latter proved not to be species-specific. Heterocytotropic antibodies are of the IgG class, in most species representing a separate sub-class or isotype of IgG. Thus, for instance,

in man IgG4 antibodies bind to mast cells, whereas antibodies of sub-classes IgG1, IgG2 and IgG3 do not.

IgE antibodies are the most important with regard to sensitisation because of their high affinity for mast cell receptors for the Fc region of the immunoglobulin FcεR. If during an immune response antigen-specific IgE antibodies are produced then they will 'prime' mast cells through interaction with FcεR. At this point the individual is sensitised and capable of mounting a hypersensitivity reaction following subsequent exposure to the same or similar substance.

If the individual sensitised in this way encounters allergen in the skin then inflammation (oedema and erythema) will result from the release by mast cells in this tissue of bioactive mediators (the basis of "skin prick tests"; see Section 5). Alternatively, if the allergen is inhaled and encountered in the respiratory tract then inflammation will occur at this site, as described below.

The critical event during the sensitisation process is the development of mast cell binding antibody, and in particular IgE. In recent years much has been learned about the cellular and molecular events which initiate and control IgE responses. A number of suppressor mechanisms have been described which apparently inhibit IgE antibody production. Many of these remain ill-defined and their relevance to the induction and regulation of IgE responses under physiological conditions is uncertain (Katz, 1980; Ishizaka, 1982; Sorg, 1989).

More recently it has become apparent that cytokines play a role of particular importance in the regulation of antibody responses and in determining which isotypes of antibody are produced (Coffman *et al*, 1988). Of particular relevance to the induction of allergic disease is the fact that certain cytokines reciprocally regulate IgE antibody production.

Interleukin 4 (IL-4) and interferon γ (IFN-γ) are the most important cytokines with respect to the regulation of IgE antibody. It is known that, in mice, the initiation and maintenance of IgE responses is dependent upon the availability of IL-4 (Finkelman *et al*, 1988a). In contrast, IFN-γ inhibits IgE production in the mouse (Finkelman *et al*, 1988b). In man also IL-4 and IFN-γ have similar reciprocal effects on IgE antibody (Del Prete *et al*, 1988; Pene *et al*, 1988; Romagnani *et al*, 1989).

Interestingly, there exists a functional heterogeneity among T helper (T_H) cells, the class of T lymphocytes required for B lymphocytes to respond productively to antigen and develop into antibody-producing plasma cells. Mosmann *et al* (1986) described two populations of T_H cells in mice, designated T_{H1} and T_{H2}, which differ with respect to the spectrum of cytokines they produce following activation. Although both populations secrete interleukin 3 (IL-3) and granulocyte/macrophage colony-stimulating factor (GM-CSF), only T_{H1} cells produce interleukin 2

(IL-2), tumour necrosis factor β (TNF-β, lymphotoxin) and IFN-γ and only T_{H2} cells produce interleukins 4, 5, 6 and 10 (IL-4, IL-5, IL-6 and IL-10) (Mosmann and Coffman, 1989). There is no doubt therefore, that the selective activation of functional subpopulations of T_H cells and the consequential selective production of regulatory cytokines will have a significant impact on IgE responses and sensitisation. The development of respiratory sensitisation will be favoured by T_{H2} cell activation.

Heterogeneity among T_H cells is not restricted to the mouse. Recently a similar functional dichotomy of human T_H cells has been confirmed (Romagnani, 1991), and there is emerging evidence that immediate-onset allergic reactions in man are associated with the selective activation of T_{H2}-type cells (Parronchi *et al*, 1991).

It appears likely therefore that the nature of T cell activation and the relative availability of specific cytokines, particularly IL-4 and IFN-γ, in the immunological microenvironment will be of critical importance in determining the initiation of IgE responses. Conditions which favour the activation of T_{H2} cells and IL-4 production will facilitate IgE antibody responses and the development of respiratory sensitisation. Variables which might affect the induction of T_{H2}-type responses (rather than T_{H1}-type responses) include the nature of the antigen, the route and duration of exposure, genetic predisposition and possibly environmental factors.

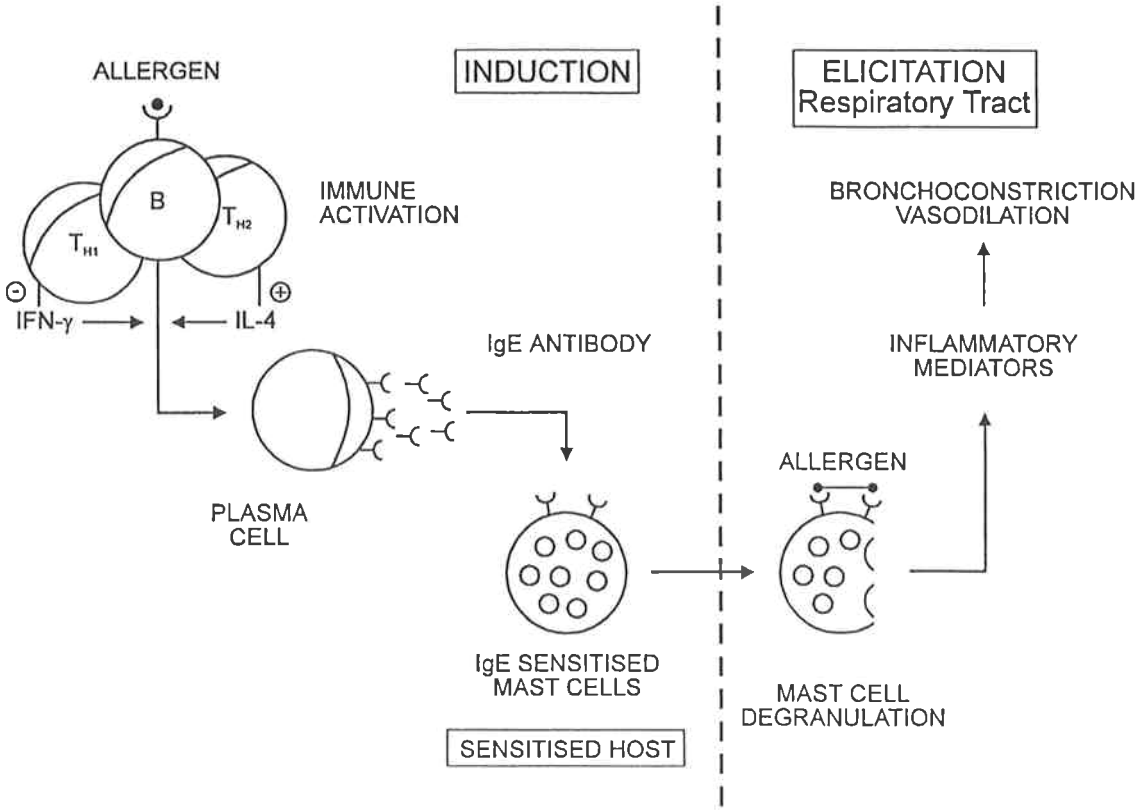
IgE antibody induced by exposure to respiratory allergens will prime mast cells which are found throughout vascularised tissue. When the same antigen is encountered for a second time then antibody primed mast cells may be induced to degranulate. The antigen associates with mast cell-bound IgE, cross-links the antibody and causes membrane perturbation and the release of various inflammatory mediators. These are many and varied and include:

- vasoactive amines (such as histamine) which induce increased vasopermeability, exudation of plasma and oedema, and
- cyclo- and lipoxygenase products of arachidonic acid metabolism, some of which induce the contraction of smooth muscle.

When these events occur in the respiratory tract of sensitised individuals, clinical signs ranging from wheezing and rhinitis to frank asthmatic reactions are manifest (Figure 1).

There is a growing interest in the possibility that cell-mediated immune processes may play a role in the pathogenesis of asthma (Corrigan and Kay, 1992). Chronic inflammation plays an important role in asthma and is associated with the accumulation of leukocytes in the bronchial mucosa, the production of mucus, the destruction and sloughing of airway cells and subepithelial fibrosis

FIGURE 1 INDUCTION AND ELICITATION OF RESPIRATORY ALLERGY

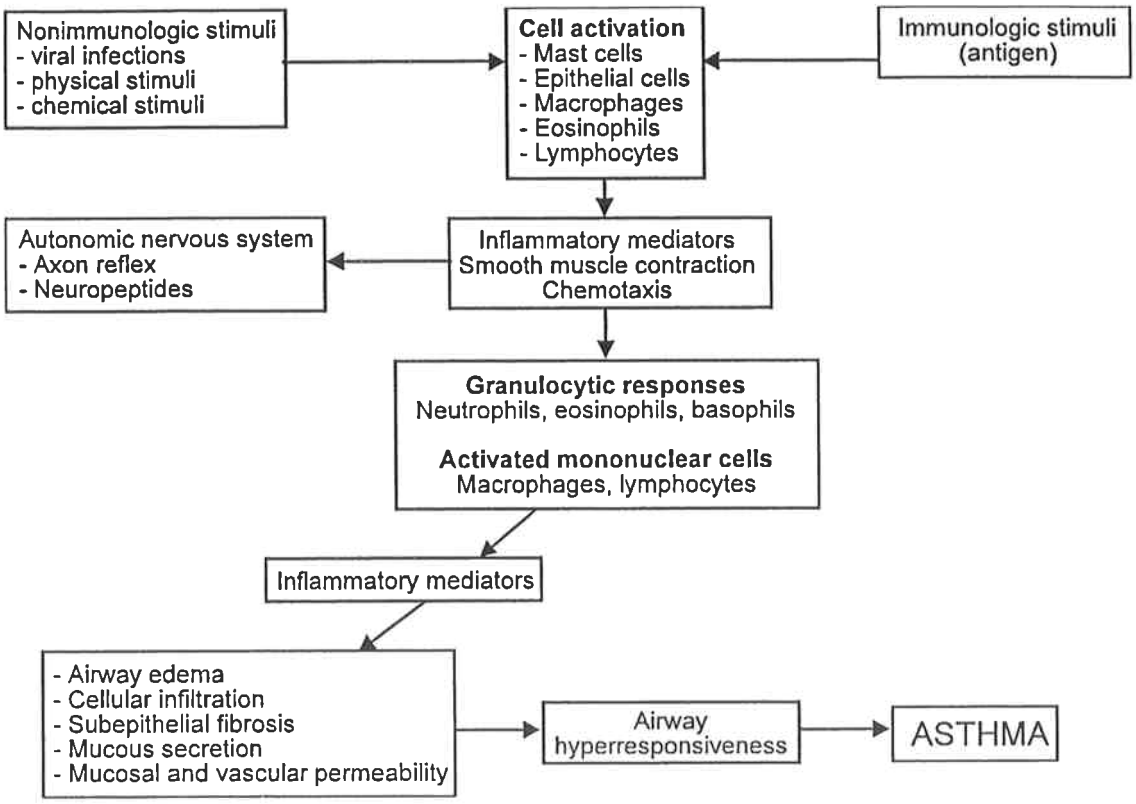


secondary to collagen deposition. Central to the development of chronic bronchial inflammation and injury are T lymphocytes acting together with eosinophils. Cell mediated immune reactions may be important in the development of late phase respiratory reactions to chemical allergens and in the longer-term development of asthma.

4.3 THE ROLE OF AIRWAY HYPERRESPONSIVENESS

Asthma is characterised by airway hyperresponsiveness, a condition manifested by an exaggerated bronchoconstrictor response to many physical changes and chemical and pharmacological agents (Boushey *et al,* 1980). Asthma patients develop clinical symptoms after exposure to allergens, environmental irritants, viral infections, cold air, or exercise. Airway hyperresponsiveness also appears to be important in the pathogenesis of asthma, as it is ubiquitous in the disease (Bleecker, 1985). Several mechanisms have been proposed to explain airway hyperresponsiveness in asthma, including airway inflammation, abnormalities in bronchial epithelial integrity, alteration in autonomic neural control of airways, changes in intrinsic bronchial smooth muscle function, and baseline airflow obstruction (Sheffer, 1991a). Proposed pathways in the pathogenesis of bronchial inflammation, airway hyperresponsiveness and asthma are summarised in Figure 2.

FIGURE 2 PROPOSED PATHWAYS IN THE PATHOGENESIS OF BRONCHIAL INFLAMMATION, AIRWAY HYPERRESPONSIVENESS AND ASTHMA (Sheffer, 1991a)



SECTION 5. RESPIRATORY SENSITISATION IN MAN

5.1 DIAGNOSIS OF INDIVIDUAL CASES

The diagnosis of allergic asthma (Sheffer, 1991a) and rhinitis is based upon a clinical history, a physical examination, lung function and immunological tests as well as bronchial or nasal provocation tests. A detailed description of the diagnostic criteria applied to these tests is given in Appendix B. The main aspects of these criteria are given below.

5.1.1 Clinical History

A complete clinical history is an important part in the diagnosis of asthma, especially in the investigation of occupational asthma. Emphasis is given to occupational and family history, type and pattern of symptoms and nature of the associated precipitating and/or aggravating factors. The diagnosis will then be established on the basis of supplementing objective measurements (Malo *et al,* 1991).

5.1.2 Physical Examination

A physical examination for asthma should focus on the upper respiratory tract and the chest. Relevant findings may include presence of rhinitis and/or sinusitis and evidence of hyperinflation of the lungs and wheezing.

5.1.3 Lung Function Tests

The most commonly used lung function measurements are the peak expiratory flow rate (PEFR), forced expiratory volume in one second (FEV1), forced vital capacity (FVC) and maximum midexpiratory flow rate (MMEF). PEFR measures the maximum flow rate generated during a forced expiratory manoeuvre, FEV1 measures the volume of air expired in one second at maximum expiration, FVC measures the total volume of air expired as rapidly as possible and MMEF measures the slope of the line between 25% and 75% of FVC.

Monitoring the PEFR can be performed by a portable instrument. Measurements and recordings 2 - 8 times a day of the PEFR for 2 - 4 weeks has proven to be a valuable tool in the diagnosis of asthma.

5.1.4 Immunological Tests

The demonstration of specific IgE antibody is a necessary but not sufficient criterion to establish the diagnosis of allergic asthma or rhinitis because the presence of specific IgE antibody is not unique to clinically allergic individuals. The presence of specific IgE antibody can be detected by *in vivo* tests such as skin prick test and *in vitro* tests such as radioallergosorbent test (RAST) or enzyme-linked immunosorbent assay (ELISA). Other *in vitro* tests, such as histamine release from basophils, are less standardised. When allergens are not available, histamine release from basophils can be an alternative to direct measurement of IgE.

5.1.5 Bronchial Provocation Tests

The degree of bronchial hyperresponsiveness can be assessed by challenging the individual with non-specific stimuli such as cold air, histamine or methacholine. In addition, provocative challenge with the suspected causative allergen can be used to confirm the diagnosis of allergic asthma. Lung function is assessed before and after the bronchial challenge. Specific bronchial challenges represent an important method, if available, to confirm a diagnosis of occupational asthma in an individual worker and to determine the causative agent (Pepys and Hutchcroft, 1975). They are indicated in studying a previously unrecognised cause of occupational asthma and in determining the precise etiologic agent. Furthermore they can be used for research on mechanisms of occupational asthma in fully informed subjects and with the approval of an ethics committee. Specific bronchial challenge should be avoided when a subject with history of occupational asthma has

- objective confirmation of asthma and work-related bronchoconstriction,
- exposure to a well-known occupational asthma agent and
- confirmation of sensitisation to this agent (specific IgE antibody detected by immunological tests).

5.2 EPIDEMIOLOGICAL APPROACH TO THE DETECTION OF IMPORTANT SENSITISERS

Asthma morbidity and mortality are on the rise. From 1980 to 1987, the prevalence rate of asthma in the United States increased by 29 %, and death rates with asthma as the first-listed diagnosis increased by 31 % (Lenfant, 1991). It is estimated that 2 % of all asthma may be of occupational origin (Chan-Yeung, 1990). Few large surveys are available, but the incidence of occupational

asthma ranges from approximately 4 % to 10 % among people exposed to laboratory animals to 44 % among workers in small bakeries (Sheffer, 1991b).

In the absence of predictive tests, substances capable of inducing respiratory sensitisation are usually identified by clinical case reports. Although a positive bronchial provocation test with the suspected material is generally considered to be the "gold standard" for the identification of a respiratory sensitiser in individual patients (WHO, 1986; Cartier *et al*, 1989), it is not routinely employed in epidemiological studies. The temporal relation between work and complaints, standard lung function tests, positive skin prick tests with the material or its protein conjugate and the demonstration of specific IgE in the serum are all used as elements of proof, either singly or in all possible combinations (Grammer *et al*, 1989).

Case reports may serve as an adequate hazard identification, but not as a risk estimate, because data on route and extent of exposure and on the "population at risk" usually are lacking. Nevertheless, for most of the substances classified as respiratory sensitisers, only case reports are available. In the absence of case reports, it is not possible to conclude that there exists no potential for sensitisation. Substances on which no case reports are available are seldom if ever subjected to further study.

Ideally, a risk evaluation is based on a longitudinal study in a population of sufficient size; it should start at the beginning of the exposure and continue until the incidence rate (number of new cases/size of population-at-risk in a given time period) has stabilised; routes and extent of exposure must be fully documented; full specific and non-specific diagnostic procedures must be followed with every member of the population and particularly with the drop-outs; a comparable control group must be studied at the same time if the diagnostic tools are non-specific. For practical and ethical reasons, this counsel of perfection is hard to follow.

Instead, case reports are sometimes followed by a cross-sectional study of the total or a part of exposed population. Examples of substances thus studied are proteolytic enzymes, phthalic and trimellitic anhydrides, platinum salts and isocyanates. The high percentages of sensitised and symptomatic workers in these early studies indicated a substantial risk under uncontrolled exposure to these agents (Gilson *et al,* 1976). However, it is not possible to be more quantitative, as data on the initial population at risk, control groups and exposure data are lacking. Semi-quantitative comparisons are also very difficult due to different diagnostic criteria. Skin prick tests are generally used as an indicator of sensitisation, but the criteria for allergic disease are as variable as the case reports.

Furthermore, the outcome of a cross-sectional study may be either an underestimate or an overestimate of the "true" risk due to two opposing factors. One is the self-selection mechanisms operating in a workforce exposed to disease-producing agents. This results in loss of "cases" and an underestimate of risk. The other is the lack of historical data on extent and routes of exposure. By the time an epidemiological study is set up, working practices may already have been improved such that the levels of exposure recorded are lower than those associated with the initial outbreak of the disease (Hallenbeck, 1988).

In a few cases, the technical measures to reduce exposure were checked with longitudinal surveys of the workforce. Thus, both in the handling of proteolytic enzymes and in the manufacture of diisocyanates, a drastic reduction of inhalatory exposure was successful in controlling the occupational health problem (Gilson *et al*, 1976). In the case of toluene diisocyanate (TDI), a threshold for sensitisation has been derived. However, in those instances where pre-employment screening has been used, a selection bias may have been created.

Quite likely the attention drawn to the sensitising capacities of these chemicals led to increased awareness and more careful handling, thus reducing the chance of exposure by all routes. As with case studies, the absence of epidemiological studies on a given substance is neither proof of absence of a sensitisation hazard nor of negligible risk.

In summary, epidemiology has provided some semi-quantitative estimates of risk in the case of exposure to sensitising agents, but has not demonstrated the absence of risk. As the epidemiological answer comes with considerable delay, accurate predictive methods are urgently needed.

SECTION 6. HAZARD IDENTIFICATION

6.1 INTRODUCTION

The prospective identification of chemicals and proteins which have the potential to induce respiratory sensitisation is still in its infancy. There is, as yet, no widely-applied or fully validated method available.

Although certain classes of chemicals such as acid anhydrides and isocyanates are associated with occupational respiratory allergy, there is presently insufficient information available to predict respiratory sensitisation potential from analysis of structure alone (see Section 6.4). Some physicochemical characteristics and biological properties appear to be important correlates of respiratory sensitisation. Reactivity with proteins and lipid solubility are likely to be relevant and it is probable that most, if not all, chemical respiratory allergens also have the potential to cause skin sensitisation in experimental models. It is clear, however, that very few of the chemicals which elicit positive responses in predictive tests for contact sensitisation have been found to induce respiratory allergy in man. Effective use of structure-activity relationships for the prospective identification of respiratory sensitisers will not become a reality until more is known of this disease and the activity of different chemical classes established.

Two main approaches to hazard identification exist: the use of animal models and *in vitro* methods. In the case of animal models both mice and guinea pigs have been used. In the main, work in mice has focused upon events occurring during the induction phase of sensitisation following primary encounter with the test chemical. In contrast, guinea pig methods seek to identify respiratory allergens as a function of elicitation reactions induced in previously sensitised animals.

6.2 ANIMAL MODELS

6.2.1 Mouse Model

There has been only a single systematic attempt to develop a method for the prospective identification of chemical respiratory allergens in mice. In this method events occurring during the induction phase, rather than during the elicitation phase of respiratory sensitisation, are measured.

This method has as its theoretical basis the fact that chemical allergens of different classes induce qualitatively different immune responses in mice. Dearman and Kimber (1991) and Dearman *et al* (1991; 1992a) performed experiments with trimellitic anhydride (TMA) and 2,4-dinitrochlorobenzene (DNCB). TMA is a well known human respiratory allergen and a comparatively weak contact

allergen. In contrast, although it is a strong contact sensitiser, DNCB apparently lacks the potential for respiratory sensitisation. Although both chemicals were immunogenic in mice, they induce qualitatively different immune responses characteristic of differential activation of functional subpopulations of T helper cells (T_{H1} and T_{H2} cells, see Section 4). TMA induces responses characteristic of T_{H2} cell activation including both an increase in the serum concentration of IgE and the appearance of hapten-specific IgE antibodies. DNCB was found to stimulate selectively T_{H1}-type responses and failed to cause either an increase in serum IgE concentration or the production of IgE anti-hapten antibody (Dearman and Kimber, 1991). Subsequent studies have revealed that exposure of mice to other known human respiratory allergens such as diphenylmethane diisocyanate (MDI) and phthalic anhydride (PA) also results in T_{H2}-type responses and IgE production (Dearman and Kimber, 1992; Dearman *et al*, 1992b). Conversely, other chemicals which, while contact allergens, are known or expected not to possess respiratory sensitising potential (4-ethoxymethylene-2-phenyloxazolin-5-one = oxazolone and dicyclohexylmethane-4,4-diisocyanate = HMDI) induced responses characteristic of selective T_{H1} cell activation (Dearman and Kimber, 1992; Dearman *et al*, 1992b).

Among several qualitative immune variables recorded, a consistent finding was that only those chemicals (TMA, PA, MDI) which have the potential to cause respiratory allergy in man provoke a significant increase in the serum concentration of IgE in mice; a consequence presumably of the production by activated T_{H2} cells of interleukin 4 (IL-4), an inducer of IgE production (see Section 4). Conversely, contact allergens which are considered to lack the potential to induce respiratory allergy in man (DNCB, HMDI, oxazolone) fail to increase mouse serum IgE concentration.

On the basis of these data, a novel predictive test method, the Mouse IgE Test, has been described wherein respiratory sensitising potential is measured as a function of increases in the serum concentration of IgE following topical exposure of BALB/c strain mice to immunogenic concentrations of the test chemical (Dearman *et al*, 1992c). At present this Test is being validated further with a wider range of chemicals.

6.2.2 Guinea Pig Models

Guinea Pig Models for Elicitation of Respiratory Hypersensitivity

Guinea pig models for measuring both immediate- and delayed-onset respiratory hypersensitivity reactions have been described. Guinea pigs can be sensitised in such a way as to display respiratory reactions similar to those seen in human allergic disease. The guinea pig model of respiratory anaphylaxis has been claimed to bear a close mechanistic similarity to human asthma (Drazen, 1977). In the case of irritant chemicals (e.g. certain isocyanates), guinea pigs have been

reported to respond in a similar way as observed in man regarding respiratory tract irritation, antibody production and pulmonary sensitisation (Karol, 1988). Therefore, the results of studies using this species can be considered as a suitable basis for hazard assessment in man. Of course, in order to assess the specific risk additional parameters such as the actual exposure concentration and the likelihood of exposure to high concentrations must also be taken into account. However, there may be some concern regarding the difference between human beings and guinea pigs with respect to the classes of antibodies responsible for respiratory hypersensitivity, in so far as IgG antibodies are of greater importance than IgE antibodies in immediate allergic reactions in the guinea pig.

For many years, efforts have been directed toward development of an animal model for inhalation sensitisation where exposure to foreign materials occurs via the respiratory tract. The first animal model for respiratory hypersensitivity using inhalation exposure for sensitisation and elicitation was developed using natural products, such as dander, moulds and spores (Ratner *et al*, 1927; Ratner, 1939). Using inhalation exposure, sensitisation with natural products has also been achieved in several other animal species (Karol, 1981a). In the guinea pig characteristic respiratory reactions attributable to respiratory hypersensitivity have been described, i.e. the immediate-onset and delayed-onset respiratory response as well as the hyperreactive airway response due to respiratory tract irritation (Karol and Thorne, 1988). The pharmacological aspects of the immediate-type hypersensitivity in commonly used laboratory animals have been reviewed (Ahlstedt *et al*, 1983; Fügner, 1985; Wanner and Abraham, 1982). The guinea pig models represent useful tools to study some pathophysiological aspects of respiratory hypersensitivity as long as the limitations of the model are recognised.

Different animal models have been used to evaluate the relationship between exposure and elicitation of immediate- and delayed-onset responses of respiratory hypersensitivity to inhaled industrial chemicals (haptens), protein-conjugates of haptens or naturally occurring proteins. Pharmacological stimuli (e.g. aerosolised histamine) have also been used (Table 2) to substantiate the findings observed during the hapten challenge.

As summarised in Table 2, sensitisation to low molecular weight chemicals has been achieved via single or repeated inhalation exposures using the free chemical or the protein conjugate of the hapten. It has been demonstrated that sensitisation by inhalation was a concentration dependent rather than a dose (concentration x time) dependent phenomenon (Karol, 1983; Pauluhn and Eben, 1991). Experimental evidence suggests that bronchial hyperreactivity may also be related to airway mucosal injury and concomitant inflammatory reactions as a result of exposures to irritant concentrations of chemicals used for sensitisation (Cibulas *et al*, 1986).

TABLE 2 PROTOCOLS EMPLOYED FOR SENSITISATION AND ELICITATION OF RESPIRATORY HYPERSENSITIVITY OF INDUSTRIAL CHEMICALS OR PROTEIN CONJUGATES OF INDUSTRIAL CHEMICALS IN GUINEA PIGS.

- Sensitisation by inhalation -

Substance/Exposure	Elicitation/Challenge	Reference
2-Isocyanatoethyl methacrylate-conjugate Expos.:10 min/d, 5x/week, 23 days,except day 15-19	conjugate: immediate-onset responses after approx. 2 weeks	Mullin *et al* (1983)
2-Isocyanatoethyl methacrylate-conjugate Expos.:10 min/d, 5x/week, 23 days,except day 15-19	hapten: delayed-onset responses (day 12 and 23), conjugate: immediate-onset responses (day 12-23)	Mullin *et al* (1983)
p-Tolylisocyanate (TMI)-, Hexylisocyanate (HMI)-conjugates Expos.: 10 min/d, 5x/week, up to 15 days	conjugate: immediate-onset responses on day 9 or 15, respectively.	DeCeaurriz *et al* (1987)
Hexylisocyanate (HMI)-conjugate, Expos.: 10 min/d, 5x/week, up to 18 days	conjugate: immediate-onset responses on day 10 and thereafter	Karol *et al* (1979)
Hexamethylenediisocyanate - trimer (HDI-trimer) Expos.: 3 h/d, 5x/week, 5 days	hapten (week 3), conjugate (week 4): neither immediate- nor delayed-onset responses	Pauluhn and Eben (1991)
Toluene diisocyanate (TDI) Expos.: 3 h/d, 5x/week, 5 days or up to 70 days	conjugate: immediate-onset responses	Karol (1983)
Toluene diisocyanate (TDI) Expos.: 3 h/d, 5x/week, 5 days	conjugate: immediate-onset response with the 'Karol-conjugate' but no response with the 'Botham-conjugate'	Botham *et al* (1988)
Toluene diisocyanate (TDI), Expos.: 4 h/d, 5 consecutive days	increased doses of aerosolized histamine: positive immediate-onset response	Cibulas *et al* (1986)
Toluene diisocyanate (TDI), Expos.: 3 h/d, 5 consecutive days	hapten (for 30 min, days 15-22): no immediate-onset response	Karol (1980)
Toluene diisocyanate (TDI), Expos.: 3 h/d, 5 consecutive days	conjugate (week 4): immediate-onset response	Sarlo and Clark (1992)
Trimellitic anhydride(TMA), Dichlorotriazine reactive dye Expos.: 3 h/d, 5 days	conjugate: no immediate-onset response	Botham *et al* (1988)
Diphenylmethane 4,4'-diisocyanate (MDI), Expos.: 3 h/d, up to 3 or 5 consecutive exposures	hapten (day 17,24):delayed-onset responses, conjugate (day 30): immediate- and delayed-onset responses	Karol and Thorne (1988)
p-Tolyl isocyanate (TMI) Expos.: 5 consecutive days: I) Alum-aerosol (30 min), followed by different TMI exposures II) Same as I) but without alum pre-exposure	hapten: no immediate-onset response; conjugate: positive response. Responses in alum pretreated and non pretreated animals did not differ	Karol (1980)
Phthalic anhydride (PA), Expos.: 3 h/d, 5 consecutive days	conjugate (week 4): immediate-onset response	Sarlo and Clark (1992)
Azodicarbonamide Expos.: 6 h/d, 5d/week for 4 weeks	hapten and histamine challenge (week 4): neither challenge resulted in specific reactions	Gerlach *et al* (1989)

The data summarised in Tables 2 and 3 have been selected to display the range of protocols which have been used to investigate allergic hypersensitivity. It must be emphasised that such lists are not comprehensive and do not necessarily include unsuccessful attempts to induce or elicit respiratory reactions.

As summarised in Table 3, sensitisation to low molecular weight chemicals has also been achieved via single or repeated intradermal or subcutaneous injection using the free chemical. Attempts have been made to standardise this animal model by using subcutaneous or intradermal routes of induction as the primary encounter with the test chemical (Botham *et al*, 1988; Pauluhn and Eben, 1991; Sarlo and Clark, 1992). Induction by the dermal route minimises the risk of non-specific bronchial hyperreactivity complicating the interpretation of elicitation-induced alterations.

The common features of the animal models described in Tables 2 and 3 are the measurement of elicitation of hypersensitivity during or following inhalation challenge in an attempt, in most instances, to simulate conditions of human inhalation exposure and the ensuing respiratory hypersensitivity response. Through the use of these models, the importance of the exposure concentration in the elicitation of respiratory hypersensitivity has become apparent.

A cardinal feature of the asthmatic state is the presence of airway hyperreactivity recognised as responses to lower than normal amounts of inhaled histamine (Cibulas *et al*, 1986; Griffiths-Johnson and Karol, 1991). However, respiratory effects caused by nonspecific irritation and respiratory effects resulting from challenge exposures to the free chemical are physiologically indistinguishable. In the majority of experiments summarised in Tables 2 and 3 the onset of respiratory hypersensitivity response was 'immediate' with reactions occurring either during or shortly after the challenge period. Although animals were monitored continuously up to 24 hours after challenge, only in very rare instances were delayed-onset or dual responses reported.

Parameters Monitored for the Detection of Respiratory Hypersensitivity

Breathing Parameters. Several breathing parameters that have proven to be of value in the quantitative evaluation of respiratory hypersensitivity responses in animal models are breathing frequency, flow-volume loops, respiratory minute volume, inspiratory and expiratory times, peak expiratory flow rates, tidal volume or plethysmographic pressure (Thorne and Karol, 1988; Pauluhn and Eben, 1991). The analysis of inspiratory and expiratory times as well as the presence of characteristic breathing patterns appeared to be useful to distinguish between effects caused by irritation and hypersensitivity. Two-chambered plethysmographic systems used for measuring common respiration parameters as well as specific airway conductance in non-cannulated guinea pigs have also been used (Gerlach *et al*, 1989).

TABLE 3 PROTOCOLS EMPLOYED FOR SENSITISATION AND ELICITATION OF RESPIRATORY HYPERSENSITIVITY OF INDUSTRIAL CHEMICALS OR PROTEIN CONJUGATES OF INDUSTRIAL CHEMICALS IN GUINEA PIGS

- Sensitisation by intradermal/subcutaneous injection -

Substance/Exposure	Elicitation/Challenge	Reference
Trimellitic anhydride(TMA), intradermal induction: 6 x during 5 days	hapten (week 3): immediate-onset response, conjugate challenge (week 4): immediate-onset response	Pauluhn and Eben (1991)
Trimellitic anhydride(TMA), single intradermal induction	hapten and conjugate: immediate-onset response after both challenges	Botham *et al* (1989)
Hexamethylene diisocyanate - trimer (HDI-trimer), intradermal induction: 6 x during 5 days	hapten (week 3), conjugate (week 4): neither immediate- nor delayed-onset responses	Pauluhn and Eben (1991)
Toluene diisocyanate (TDI), subcutaneous induction: 2x/week for 4 consecutive weeks, week 6: booster injection	conjugate (week 8, intratracheally): immediate-onset responses	Sarlo and Clark (1992)
Phthalic anhydride (PA) subcutaneous induction: 2x/week for 4 consecutive weeks, week 6: booster injection	conjugate (week 8, intratracheally): immediate-onset responses	Sarlo and Clark (1992)

The non-specific nature of changes in breathing frequency necessitates careful interpretation. It is widely recognised that chemicals cause a decrease in breathing frequency by sensory irritation (e.g. TDI, TMI) or an increase in breathing frequency as a consequence of pulmonary irritation and stimulation of nerve receptors (e.g. MDI) (Karol, 1991). In guinea pigs high concentrations of respiratory tract irritants can cause bronchoconstriction. To detect a characteristic immediate-onset response attributable to respiratory tract allergic hypersensitivity, the irritation response must be avoided by using sub-irritant concentrations of the free chemical, and by careful comparison of sensitised animals with control animals. A dependence of immediate-onset respiratory hypersensitivity response on the quality of the protein conjugate has also been reported (Botham *et al*, 1988).

Experimental evidence suggests that the presence or absence of a characteristic immediate-onset breathing pattern, not spontaneously occurring in control animals, is of greater diagnostic validity than the evaluation of changes in respiratory rate alone.

Antibodies. The induction of respiratory hypersensitivity is dependent on the induction of specific homocytotropic antibodies. Measurement of antibody, rather than pulmonary responses, has two potential advantages. Firstly, from a practical point of view, serological measurements may provide

evidence of respiratory sensitisation potential without the need for inhalation challenge. Secondly, antibody responses may be of more sensitive than respiratory reactions. That is, chemicals and proteins may induce the production of relevant antibodies at exposure concentrations below those which are required for measurable respiratory reactions following inhalation challenge.

6.2.3 Advantages and Disadvantages of the Mouse IgE Test and Guinea Pig Methods for Identification of Respiratory Allergens

Mouse IgE Test

The advantages of the mouse IgE test are that:

- it uses fewer animals and does not depend upon the development of an adverse reaction,
- it is relatively rapid and cost-effective and comparatively easy to perform,
- it is quantitative and relevant in that it is based upon measurement of IgE, the class of antibody known to effect immediate hypersensitivity reactions,
- dose-response relationships at the induction phase can be measured; the relative antibody producing potential of chemicals can be determined and no-observable-effect levels (NOEL) for IgE antibodies established.

Disadvantages of the mouse IgE test are that:

- it is not at present suitable for measuring the respiratory sensitisation potential of proteins,
- it cannot be used for evaluation of cross-reactivity between chemical respiratory allergens,
- it has not been formally validated.

Guinea Pig Methods

The advantages of the guinea pig methods described above are that:

- respiratory hypersensitivity is measured as a function of elicitation reactions during or following inhalation challenge exposure, the relevant route for the elicitation of pulmonary reactions in man,
- elicitation reactions can be measured using a variety of breathing parameters,
- induction can be performed using a variety of exposure routes,
- dose/concentration response studies can be performed at both the induction and elicitation stages and no-observable-effect-levels determined,
- cross-reactivity between respiratory allergens can be investigated,
- the respiratory sensitisation potential of proteins can be measured.

The disadvantages of guinea pig methods are that:

- they are time consuming and costly,
- they depend upon a number of test-specific factors, such as specific antibody measurements and the generation of the challenge atmosphere,
- they require specialised personnel and inhalation laboratory facilities,
- effective elicitation of sensitisation to chemicals by inhalation exposure may require the use of a hapten-protein conjugate,
- standardisation of hapten-protein conjugates is difficult to achieve; in addition, selection of the most appropriate sub-irritating inhalation challenge concentrations of free chemical is difficult and measurements of pulmonary hypersensitivity may be confounded by respiratory irritancy,
- there is no well-defined IgE antibody in guinea pigs, and standardised reagents for detailed analysis of serological responses are lacking,
- tests have not been formally validated.

6.3 IN VITRO METHODS

There are no standard validated *in vitro* methods for determining the potential of a chemical to cause respiratory sensitisation. However, the potential of a low molecular weight chemical to interact with protein can be considered a prerequisite to its ability to be an allergen, since low molecular weight compounds are incomplete antigens (haptens), which require binding to protein carriers to become immunogenic. Thus, attempts have been made to predict the immunogenicity of a chemical from its protein or peptide binding properties. A number of published studies evaluated the interaction between protein and chemicals known to cause occupational asthma, such as trimellitic anhydride, chloramine-T, β-lactam antibiotics and isocyanates (Patterson *et al*, 1978; Evans *et al*, 1986; Edwards *et al*, 1988; Jin and Karol, 1988). Using high pressure liquid chromatography, Wass and Belin (1990) demonstrated that isocyanates, anhydrides, and chloramine-T reacted with a lysine-containing peptide, whereas simple acids, bases, and solvents did not. The ability of a low molecular weight chemical to bind to protein or peptides may therefore be useful as a screen for detecting potential respiratory sensitisers, especially for new chemicals or complex mixtures with unknown properties.

However, binding studies *in vitro* are not models for the induction of an immunologic response, a complex physiologic event involving the interaction of many cell types and cytokines. No *in vitro* model of respiratory sensitisation exists which results in the production of antigen-specific homocytotropic antibodies capable of binding to tissue mast cells. An *in vitro* model would also

need to address the problems of solubility, metabolism, and direct toxicity of potential respiratory sensitisers.

There are also no *in vitro* correlates to the immediate-onset reaction (elicitation phase of the immune response) typical of many respiratory sensitisers, since these assays would require a population of cells obtained from animals following prior exposure(s) to a potential sensitising chemical. The problems associated with the site and availability of such cells, the timing of collection, and the solubility, composition, and inherent toxicity of the antigen have made these studies difficult to design and perform. Finally, the endpoints measurable *in vitro* which would correlate with endpoints observed during both immediate- and delayed-onset respiratory allergic reactions *in vivo* are not completely known given the current state of knowledge of the causative factors in each of these reactions.

In conclusion, the use of *in vitro* assays to model the inductive and elicitation phases of the immune responses in respiratory allergy rests in the future. As the mechanisms involved in respiratory sensitisation became clearer, *in vitro* assays useful for predicting potential chemical sensitisers may result.

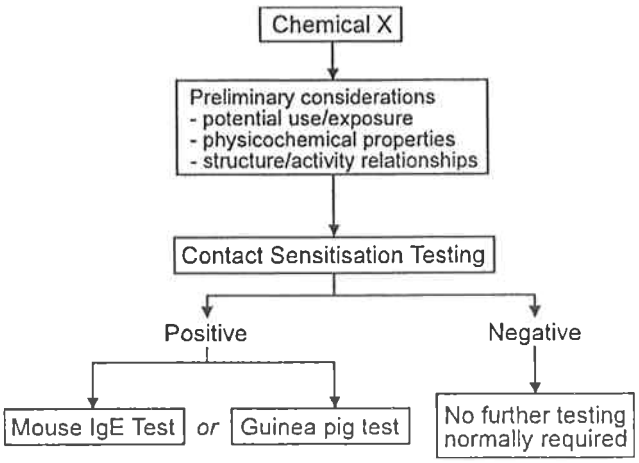
6.4 STRUCTURE ACTIVITY RELATIONSHIPS (SAR)

The ability to predict the *in vivo* activity of a chemical based upon analysis of its structure has recently been examined in the area of respiratory sensitisation. Agius *et al* (1991) generated a structure activity hypothesis from investigations comparing the structure and reactivity of substances known to cause occupational asthma with that of other related chemicals which were known not to cause occupational asthma. The ability of molecules to form multiple covalent, coordination and hydrogen bonds was identified as an important predictor of occupational asthma. This hypotheses now needs to be tested and revised. A better understanding is also needed in the area of chemical-protein interactions, since the immunological response involved in respiratory sensitisation may well be directed against chemical-induced conformational changes in carrier proteins such as albumin. Kochman *et al* (1990), using Fourier transform infrared spectroscopy of guinea pig serum albumin following repeated inhalation of the known respiratory sensitiser toluene diisocyanate, demonstrated major alterations in the alpha helix content of albumin compared to control protein. These new conformational determinants may play an important role in the development of respiratory sensitisation.

6.5 RECOMMENDATIONS FOR IDENTIFICATION OF RESPIRATORY SENSITISATION HAZARD OF CHEMICALS

It must be emphasised that there are as yet no widely accepted methods for the prospective evaluation of respiratory sensitising activity. However, based upon the evidence available a process for hazard identification of chemical respiratory sensitisation is suggested which is summarised in Figure 3. It should be noted also that this process is unsuitable for assessment of proteins for which no harmonised methods exist.

FIGURE 3 RECOMMENDED TESTING STRATEGY FOR CHEMICALS (NOT FOR PROTEINS)



6.5.1 Preliminary Considerations

Necessarily the first step in any hazard identification process should include an examination of the physicochemical properties of the test material, particularly in the context of structure-activity relationships. With regard to identification of respiratory sensitisation hazard, important considerations include the volatility of the chemical and the likelihood that aerosols or vapours will be present during manufacture or use. Certain classes of chemical (acid anhydrides and isocyanates for instance) may signal particular concern.

6.5.2 Skin Sensitisation

In the case of chemicals (other than proteins) it appears that only those materials which exhibit at least some potential to cause skin sensitisation in experimental animals are able to induce respiratory allergy. The Task Force recommends therefore that an important step in the hazard identification process is the performance of a well-conducted standard predictive testing for skin

sensitisation activity (guinea pig maximisation test, local lymph node assay etc). Chemicals which lack the potential to cause contact sensitisation can be classified also as lacking the ability to induce respiratory allergy and it is recommended that no further testing will usually be necessary. If, however, it has been possible to assess skin sensitisation potential only at low concentrations and the results were negative, then respiratory sensitisation testing may still be necessary.

6.5.3 Respiratory Sensitisation

If identification of the respiratory sensitisation hazard of chemicals is then required there exist two options, neither of which is fully validated.

Mouse Test

This method should be performed according to the preliminary protocol described elsewhere (Dearman *et al,* 1992c). It is the recommendation of the Task Force that each chemical is tested at at least three concentrations which can be selected on the basis of skin sensitisation test data.

Guinea Pig Tests

The Task Force recommends that protocols requiring inhalation sensitisation or challenge with hapten protein conjugates be avoided.

The approach favoured is the use of intradermal or subcutaneous administration for sensitisation since the site of induction and challenge are different. It also appears that the intradermal induction-inhalation challenge protocol is easier to standardise and therefore less susceptible to experimental factors when compared with the inhalation induction-inhalation challenge protocol. Confounding effects due to neurogenic inflammation cannot occur.

Challenge should be via the respiratory tract (inhalation exposure to aerosols is appropriate, but intratracheal administration has also been used successfully). Inhalation challenge with irritant vapours is inappropriate. Challenge induced reactions should be measured as a function of changes in respiratory volume and rate and breathing pattern. It is recommended also that test and control animals be examined for the presence of anti-hapten antibody. There is still no consensus regarding many detailed aspects of procedure and protocol and it is not possible presently to give clear recommendations with respect to a number of issues.

SECTION 7. RISK ASSESSMENT IN RESPIRATORY ALLERGY

In all forms of chemical and protein induced allergic hypersensitivity there are two types of risk assessment:

- the risk that exposure to a material will induce sensitisation in an individual;
- the risk that exposure to a material will elicit allergic reactions in a previously sensitised individual.

In most instances the dose of chemical required for initial sensitisation will be different from and higher than that necessary to elicit respiratory hypersensitivity reactions in a previously sensitised individual.

One part of the risk assessment process relies upon evaluation of the potency of the material. In theory, the relative potential of a chemical to cause respiratory sensitisation can be evaluated by the methods described previously in Section 6. In the case of guinea pig tests this would require estimation of the amount of material capable of inducing a given level of sensitisation as judged by either the stimulation of antibody production or by the subsequent elicitation of respiratory reactions following inhalation exposure or intratracheal administration. Alternatively, an estimation of the NOEL could be made. In the mouse IgE test the preferred approach would be to identify the minimum concentration of chemical necessary to cause a significant increase in the serum concentration of IgE.

In practice such measurements would be of value only in terms of relative potency; that is relative to the activity of a known chemical, of a similar class, for which occupational health data were available. For example it might prove possible to evaluate whether a new chemical is more or less likely than an index chemical of related structure to cause occupational respiratory sensitisation under similar conditions of exposure. If there exists an occupational exposure level for the index chemical, then such comparisons should provide one approach to determining safe working conditions for the new material.

Evaluation of the ability of a chemical to cause respiratory allergic reactions in a previously sensitised animal could be approached only by the use of guinea pig models which employ inhalation challenge. For human beings already sensitised to a given material, there is no consensus on the possibility of identifying a dose/concentration below which adverse effects are unlikely to occur.

The other aspect of risk assessment requires consideration of factors other than inherent potency. For respiratory sensitisation and the elicitation of respiratory reactions in previously sensitised individuals this includes:

- extent, frequency, duration and route of exposure (peak exposures may be particularly important);
- concomitant exposure (especially with potential adjuvants);
- genetic or acquired differences in susceptibility to sensitisation via the skin (for chemicals) or the respiratory tract (for chemicals and proteins).

SECTION 8. CONCLUSIONS AND RECOMMENDATIONS

A number of chemicals and proteins of industrial importance are known to cause respiratory allergy. There are still several gaps in our understanding of the sequence of events through which chemical agents evoke an immune response leading to specific allergic clinical manifestations. Some kinds of exogenous chemical exposure appear more likely to evoke immune reaction than others. Likewise, some chemicals affecting the human immune system appear to manifest themselves preferentially in one immune response rather than another. For proteins, which are complete antigens, the main concern is in establishing the relative allergenic potential. For low molecular weight chemicals acting as a hapten, we need to know which chemical properties are important for its binding to the autologous carrier protein, and which chemical properties are responsible for the hapten-carrier complex behaving immunogenically.

The skin and the respiratory tract are the most important organs exposed to the environment and which can be affected by immune responses to chemical agents. Evidence is emerging that the skin also may be an important route for sensitisation for respiratory allergy to chemicals. The relevant route of exposure for sensitisation to protein respiratory allergens is almost invariably by inhalation. Respiratory allergens induce a specific IgE antibody response while exposure to skin contact allergens does not.

It is also important that the large body of data pertaining to human disease is not ignored, even if they generally lack an assessment of exposure and estimation of risk. Attempts have been made to derive structure activity relationships for a wide range of chemical agents which may cause allergic asthma (Agius *et al*, 1991). Given the possible serious health manifestations of respiratory allergy, early identification of possible sensitisers is urgently required. In recent years significant advances have been made in our understanding of both the mechanisms underlying respiratory allergy, as well as the factors predisposing to it, but not to the extent needed for a rigorous risk assessment. However, the increased understanding has resulted in the development of new but as yet not fully validated predictive methods for the identification of chemicals and proteins that have the potential to cause respiratory sensitisation. A multidisciplinary approach is essential to understand the ways in which chemicals interact with the immune system to produce allergies and similar manifestations (ECETOC, 1987). In addition to the use of animal models, more emphasis has to be placed on clinical and epidemiological studies, especially of workers, with emphasis on better qualitative and quantitative measures of exposure to specific chemicals in the relevant environment (Agius, 1992).

It is concluded that for chemicals, the two methods which are the most promising with respect to predictive testing and which should, as a priority, be developed further, are the mouse IgE test and

the guinea pig model with induction by intra- and/or subcutaneous injection followed by inhalation challenge with the free chemical. The Task Force recommends that for the identification of proteins with respiratory allergenic potential, a guinea pig model should be used. Further validation of the methods will be facilitated by the production of a detailed protocol. Occupational hygiene aspects and epidemiology will need to be included in the validation process.

The further development of tiered approaches for hazard identification should be encouraged, which should include the development of SAR's.

Since both skin and respiratory sensitising chemicals can induce sensitisation through skin contact, one method of reducing sensitisation from respiratory sensitisers is to control skin contact, which should be taken into account in setting occupational hygiene standards for these chemicals.

APPENDIX A. GLOSSARY OF TERMS

ADJUVANT:

A substance which enhances or modifies the immune response.

AIRWAY HYPER-RESPONSIVENESS:

A heightened or exaggerated reaction to a variety of immunologic or nonimmunologic (cold air, exercise) stimuli resulting in bronchoconstriction ('asthma attack').

ALLERGEN:

An antigen responsible for inducing allergic reactions.

ALLERGY:

An adverse reaction mediated by an immune response usually due to an exogenous substance.

ANAPHYLAXIS:

An immediate hypersensitivity reaction, sometimes fatal, occurring in sensitised individuals following re-exposure to an allergen, which results in vasodilation and constriction of smooth muscle, including that of the bronchi.

ANTIBODY:

A protein of the immunoglobulin class produced by plasma cells in response to an antigen which has the ability to combine specifically with the antigen that induces its formation.

ANTIGEN:

A substance which can induce an immune response.

ARACHIDONIC ACID METABOLITES:

Mediators formed from the metabolism of arachidonic acid via the cyclo-oxygenase pathway (prostaglandins, thromboxanes) or lipoxygenase pathway (leukotrienes, hydroxyacids) which can be generated in lung tissue and are believed to contribute to the pathophysiology of asthma.

ASTHMA:

A lung disease characterised by variable and reversible airflow obstruction caused by bronchial constriction with oedema and secretion, followed by inflammation.

B LYMPHOCYTE (B CELL):

Lymphocytes which express membrane immunoglobulins and are the precursors of antibody-forming plasma cells.

BASOPHIL:

A blood granulocyte whose granules contain histamine, serotonin and other mediators of inflammation, which are released after cross-linking of membrane-bound IgE antibodies to the basophil Fc(epsilon) receptor.

CAPPED ISOCYANATES:

Isocyanates chemically reacted with nucleophilic compounds such as phenols or amines. The resultant urethane or urea compound lacks the ability to react with proteins. Thus, the isocyanate specific chemical reactivity (and toxicity) is "capped". The capped isocyanate is thermolytically degraded to the original isocyanate and nucleophile at higher temperatures.

CARRIER PROTEIN:

A protein with which a hapten must be associated to induce an immune response.

CYTOKINES:

A generic term for secreted proteins and glycoproteins which influence immune and inflammatory responses (e.g. IL-4, Interferon-γ).

CYTOTROPIC ANTIBODY:

Antibodies that bind by their constant (Fc) regions to receptors expressed on cell membranes.

Fc(ε)R:

Cell surface receptors with a binding affinity for IgE antibody.

FORCED EXPIRATORY VOLUME IN 1 SECOND (FEV1):

The volume of air expired in 1 second from maximum inspiration.

FORCED VITAL CAPACITY (FVC):

Total volume of air expired as rapidly as possible.

HAPTEN:

A chemical capable of binding with antibody when associated with a carrier protein but which on its own is unable to stimulate an immune response.

HETEROCYTOTROPIC ANTIBODIES:

Antibodies which can bind to cells in species other than the one in which they were produced.

HISTAMINE:

A vasoactive amine of MW 111 released from mast cells and basophil granules and which causes smooth muscle contraction of lung bronchioles and small blood vessels, increased permeability of capillaries and increased secretion by nasal and bronchial mucous glands.

HOMOCYTOTROPIC ANTIBODIES:

Antibodies which bind to cells in animals of same or similar species in which they were produced.

HYPERREACTIVITY:

See Airway hyperresponsiveness.

HYPERSENSITIVITY:

A state of heightened reactivity to antigen resulting from previous sensitisation, known also as allergy. Hypersensitivity denotes an adverse rather than a protective response.

IgE:

The major anaphylactic antibody in man and in mice.

IgG:

A class of antibody.

IL-4:

A cytokine produced by activated T cells which plays an important role in antibody responses.

INTERFERON-(γ):

A cytokine secreted by activated T lymphocytes which has pleiotropic biological properties including regulation of IgE responses.

LEUKOTRIENES:

Arachidonic acid metabolites of the lipoxygenase pathway which contribute to the airway constriction, increased vascular permeability, mucous secretion, and inflammation observed in asthma.

MAXIMUM MIDEXPIRATORY FLOW RATE (MMFR):

The slope of line between 25 % and 75 % of the forced expiratory volume.

MAST CELL:

A cell found in tissue which contains histamine, serotonin, and other mediators of inflammation, which are released after cross-linking of membrane-bound IgE antibodies.

METACHOLINE:

A chemical with bronchoconstrictor properties used in provocation tests to measure the degree of airway hyperresponsiveness.

PEAK EXPIRATORY FLOW RATE (PEFR):

The maximum flow rate that can be generated during a forced expiratory manoeuvre; measured in litres per second.

PLASMA CELL:

An antibody-producing cell which is the end-stage of differentiation of a B lymphocyte.

PLETHYSMOGRAPH:

Apparatus for measuring lung volume based upon displacement.

RAST (RADIO-ALLERGOSORBENT TEST):

A solid phase radioimmunoassay for detecting allergen-specific IgE in serum.

SENSITISATION:

An immune status resulting from an immune response to antigen which may result in a clinical hypersensitivity reaction, following a subsequent exposure to the same antigen.

SKIN PRICK TEST:

A direct skin test to detect IgE antibody (see Appendix B).

T HELPER CELL:

A functional subclass of T lymphocytes which regulate immunological responses.

T LYMPHOCYTE (T CELL):

A thymus-derived lymphocyte which participates in a variety of cell-mediated immune reactions.

APPENDIX B. CRITERIA USED IN THE DIAGNOSIS OF ALLERGIC ASTHMA

B.1 CLINICAL HISTORY

A clinical history is particularly important in the diagnosis of allergic asthma and is essential in occupational asthma since the symptoms are often not present at the time of examination. Table B.1 contains relevant topics to be included.

In the diagnosis of occupational asthma, it is essential that there is a temporal relationship between a specific sensitising agent encountered in the workplace and the occurrence of respiratory symptoms.

A thorough patient history must be taken to distinguish between

- preexisting asthma that is exacerbated by exertion of nonspecific irritant exposure to a specific sensitising substance at the workplace and
- asthma that is caused solely by exposure to a specific sensitising substance at the work site.

There is often a latent period of weeks or, in some cases, years between first exposure and the onset of symptoms. Once symptoms develop, they tend to become progressively more severe with continued exposure. Symptoms include:

- Rhinitis or ocular irritation are usually the first symptoms experienced. It may occur within minutes of exposure and may disappear after the worker leaves the workplace.
- Pulmonary symptoms may first be a cough rather than wheezing and may occur first in the evening after work or during the night.
- More typical asthma symptoms (cough and wheeze, tight chest, and dyspnea) appear with continued exposure and begin to occur in closer proximity to the work exposure.
- Symptoms clearing over weekends may be one of the first clues to a possible occupational cause of patient's asthma. In some instances, improvement over the weekend is negligible, and the symptoms only subside after 1 or 2 weeks away from work.
- Outbreaks of asthma symptoms among other workers may provide clues to the causative agent in an area of close exposure to the substance.

TABLE B.1 DIAGNOSIS OF ALLERGIC ASTHMA: RELEVANT TOPICS TO BE INCLUDED IN THE CLINICAL HISTORY (Sheffer, 1991a)

Symptoms	Cough, wheezing, shortness of breath, chest tightness, and sputum production generally of modest degree. Condition known to be associated with asthma: Rhinitis, sinusitis, nasal polyposis, or atopic dermatitis.
Pattern of Symptoms	Perennial, seasonal, or perennial with exacerbation. Continuous, episodic or continuous with acute exacerbations. Onset, duration, and frequency of symptoms (days per week or month). Day/night (circadian) variation with special reference to nocturnal symptoms.
Precipitating and/or aggravating factors	Viral respiratory infections. Exposure to environmental allergens (pollen, moulds, house-dust mite, cockroach, animal danders). Exposure to occupational chemicals or allergens. Environmental change (e.g. moving to a new home, going on holidays, and/or alteration in workplace, work processes, or material used). Exposure to irritants, especially tobacco smoke and strong odours, air pollutants (ozone, sulphur oxide and nitrous oxide), occupational chemicals, vapours, gases and aerosols. Emotional expressions: Fear, anger, frustration, crying, hard laughing. Drugs and food additives (aspirin, beta-blockers, nonsteroidal anti-inflammatory drugs, sulphites). Exercise. Endocrine factors (e.g., menses, pregnancy and thyroid diseases).
Development of Disease	Age of onset, age of diagnosis. Progress of disease (better or worse). Previous evaluation, treatment and response. Present management and response, including plans for managing acute episodes.
Profile of Typical Exacerbation	Prodromal signs and symptoms (e.g. itching of skin of the anterior neck and nasal allergy symptoms). Temporal progression. Usual management. Usual outcome.
Living Situation	Home age, location, cooling and heating (central with oil, electricity, gas, or kerosene space heating), wood-burning fireplace. Carpeting over a concrete slab. Humidifier. Description of patient's room with special attention to pillow, bed, floor covering, and dust collectors. Animals in home. Exposure to cigarette smoke, direct or side-stream, in home.

TABLE B.1 (ctd.) DIAGNOSIS OF ALLERGIC ASTHMA: RELEVANT TOPICS TO BE INCLUDED IN THE CLINICAL HISTORY (Sheffer, 1991a)

Impacts of Disease	Impact on patient: Number of emergency-department or urgent care visits and hospitalisations. History of life-threatening acute exacerbation, intubation, or oral steroid therapy. Number of school or work days missed. Limitation of activity, especially sports. History of nocturnal awakening. Effect on growth, development, behaviour, school or work achievement, and life-style.
	Impact on family: Disruption of family dynamics, routine, or restriction of activities. Effects on siblings and spouse. Economic impact.
Family History	IgE-mediated allergy in close relatives. Asthma in close relatives.
Medical History	General medical history and history of other allergic disorders (e.g. chronic rhinitis, atopic dermatitis, sinusitis, nasal polyposis, gastrointestinal disturbances, adverse reactions to food and drugs). In children, history of early life injury to the airways (e.g. bronchopulmonary dysplasia, history of pulmonary infiltrates, documented pneumonia, viral bronchiolitis, and passive exposure to cigarette smoke). In adults, cigarette smoking history. Detailed review of symptoms.

Questionnaires based on the MRC (Medical Research Council) questionnaire and the American Thoracic Society Adult Questionnaire, ATS-DLD, (Ferris, 1978) have been used as an aid in the diagnosis of asthma in epidemiological studies. However, there is no standard questionnaire for asthma and therefore the reliability and validity of modified MRC and ATS-DLD questionnaires is unknown (Smith *et al*, 1989).

B.2 PHYSICAL EXAMINATION

The physical examination for asthma focuses on the upper respiratory tract, the chest, and the skin. Relevant findings may include (Sheffer, 1991a):

- presence of rhinitis and/or sinusitis, nasal polyps;
- evidence of hyperinflation of the lungs;
- quality of breath sounds; wheezing is the characteristic breath sound of asthma; the intensity of the breath sounds in symptomatic asthma is typically reduced; a prolonged phase of forced expiration is typical of airflow obstruction;
- flexural eczema.

Because asthma is characterised by variable airways constriction, symptoms may be absent at the time of examination. In acute severe asthma the patient is normally in great distress and entirely preoccupied with the act of breathing. There is often mild cyanosis, tachypnoea and the patient uses the accessory muscles of respiration. The chest is hyperresonant and there are high pitched rhonchi with prolongation of expiration. In most cases however, the symptoms may be absent or very discrete at the time of examination, and the only finding may be discrete high pitched rhonchi at stethoscopy.

B.3 LUNG FUNCTION TESTS

Pulmonary function studies are essential for diagnosing asthma and assessing the severity of asthma in order to make appropriate therapeutic recommendations. The use of objective measures of lung function is recommended because patient symptom reports and physical examination findings often do not correlate with the variability and severity of airflow obstruction.

Results from a single lung function test have limited specificity and sensitivity for the diagnosis of asthma because variable and reversible airflow obstruction is a fundamental clinical feature of asthma. The most commonly used lung function measurements are the peak expiratory flow rate (PEFR), forced expiratory volume in one second (FEV_1), forced vital capacity (FVC) and maximum midexpiratory flow rate (MMEF) (Sheffer, 1991c). To measure PEFR, the subject breathes in to total lung capacity (TLC) and then makes a maximum expiratory effort into the peak flow meter. The flow rate is measured over the first 100 ms of exhalation. Clinically useful measurements of peak flow can be made with a simple portable meter. The characteristic pattern of diurnal variation with low recordings at night and first thing in the morning is virtually diagnostic of asthma. Readings from different peak flow meters should not be compared as different makes and models do not give consistent results (Kotses *et al*, 1984). PEFR measurements are useful in monitoring the occurrence of symptoms especially in occupational asthma where their use both at work and at home can identify changes in peak flow related to workplace exposure (Butcher and Salvaggio, 1986). Individual PEFR measurements can be compared with predicted values using the nomograms accompanying each peak flow meter but many individuals' peak flow measurements

are consistently higher or lower than the published predicted values. Therefore, it is recommended that the relative lung function of an individual be assessed by comparing the peak flow measurements taken at different times with a peak flow measurement taken in the absence of airway obstruction. Spirometric measurements such as FEV_1, FVC and MMEF are a more accurate way of measuring airway function. The subject breathes in to TLC and then exhales as rapidly as possible to residual volume (RV) into a spirometer. The spirometer records exhaled volume against time. In airways obstruction, the FEV_1 falls to a greater extent than the FVC so that the ratio between these measurement falls below the normal ratio of 75%. In cases of airways obstruction without any constriction, the FVC may be normal but can also be reduced due to severe obstruction alone. In contrast, a reduced FVC and normal flow rate is indicative of airways constriction. Isolated reduction in MMEF is characteristic of mild airflow obstruction indicating small airways disease. FEV_1 is the best guide of the degree of pulmonary disorder in acute severe asthma. If FEV_1 falls below 20% of the predicted value, ventilatory failure and CO_2 retention are liable to occur. Less widely used lung function tests are the flow-volume loop, body plethysmography, analysis of lung volumes, arterial blood gases, and chest radiography.

B.4 BRONCHIAL PROVOCATION TEST

B.4.1 Measurement of bronchial hyperresponsiveness

A fundamental characteristic of active asthma is bronchial hyperresponsiveness (Hargreave, 1987). The frank asthmatic may be identified by challenge with a single dose bronchoconstrictor stimulus such as conventional exercise test, or hyperventilation to a set level of cold dry air. However, this sort of challenge is not sensitive enough to discriminate between the various stages from marked hyperresponsiveness to unresponsiveness.

A more sensitive challenge test is the bronchoconstrictor test using incremental doses of methacholine or histamine administered by inhalation at fixed time intervals. Lung function is normally assessed between doses by measuring FEV_1. The challenge sequence is continued until a predetermined level of bronchoconstriction has been achieved which is normally a 20% fall in FEV_1. By linear interpolation from the line joining the last two points on the dose response curve, the theoretical provoking dose that would have caused exactly a 20 per cent fall in FEV_1 is determined. This is termed the PD20 which is derived as a logarithmic term from a log dose response plot, but is conventionally expressed arithmetically as the anti log (Raffle, 1987). Asthmatics normally respond with a greater degree of airway obstruction at lower cumulative doses of methacholine or histamine than do normal subjects.

B.4.2 Specific bronchoprovocation test

Exposure of the sensitised individual to the relevant allergen can result in a spectrum of bronchial responses including an immediate response, a delayed response or an immediate followed by a late response (Hargreave, 1989). An immediate response begins within minutes of exposure and normally resolves within one hour. The late response begins one or more hours after exposure, reaches a peak after several hours and sometimes takes weeks to fully resolve.

In this test the subjects' airways are challenged with incremental doses of the suspect inhalant allergen and the lung function monitored by measuring PEFR, FEV_1 and/or nonspecific bronchial hyperresponsiveness. The challenge exposure can be carried out blind or double blind. If there is a possibility of a late reaction, each increment must be given on a separate day, and a period of 24 hours provided for monitoring the effects. If there is no reason to suspect a late reaction, it may be reasonable to administer incremental challenges at intervals of a few minutes (5-30 minutes). Baseline FEV_1 levels are measured at intervals before and after the challenge exposure. A sustained decrease in FEV_1 of at least 20% from the initial baseline measurement is considered a positive result.

Bronchoprovocation tests are potentially hazardous and therefore should only be carried out under the supervision of experienced physicians in properly equipped medical centres. While bronchoprovocation tests are considered the "gold standard" for confirming the diagnosis of occupational asthma, further validation is required for individual substances prior to their routine use (Cartier *et al*, 1989). The challenge concentration, method and duration of administration, and inadvertent use of drugs can influence the results of a bronchoprovocation test.

B.5 IMMUNOLOGICAL TESTS

The presence of specific IgE antibody can be detected by *in vivo* and *in vitro* tests. The most widely used *in vivo* test is the skin prick test and the most widely used *in vitro* test is the radioallergosorbent test (RAST). Skin prick tests and RAST have been extensively used as aids in the evaluation of allergy to common environmental allergens such as grass pollen and house dust mite and various occupational allergens. Examples of the various types of occupational allergens evaluated with the help of the skin prick test or RAST are given in Table B.2. The selection of appropriate allergen samples is important for both the *in vivo* and *in vitro* tests (Bush and Kagen, 1989; Bernstein and Zeiss, 1989). For low molecular weight allergens that are probably acting as haptens it is often essential to link the low molecular weight substance to a carrier protein like human serum albumin, and then to use the hapten carrier conjugate as the source of allergen.

TABLE B.2 EXAMPLES OF THE USE OF SKIN PRICK TEST OR RAST IN THE EVALUATION OF OCCUPATIONAL ALLERGENS

Allergen	Skin prick test	RAST
Bacillus subtilis enzymes	Juniper *et al*, 1977	How *et al*, 1978
Nickel	Malo *et al*, 1982	Malo *et al*, 1982
Papain	Novey *et al*, 1980	Novey *et al*, 1980
Reactive dyes	Alanko *et al*, 1978	Alanko *et al*, 1978
Phthalic anhydride	Grammer *et al*, 1987	Maccia *et al*, 1976
Platinum	Pickering, 1972	Murdoch *et al*, 1986
Toluene diisocyanate	Butcher *et al*, 1977	Baur *et al*, 1984
Acid anhydrides	Zeiss *et al*, 1977	Welinder *et al*, 1990

The skin prick test is the most commonly used *in vivo* tests in the diagnosis of immediate type allergy. This test detects IgE antibody bound to mast cells or basophils in the skin. If allergen cross links IgE antibody on the surface of these cells then mediators such as histamine are released which result in the classic weal and flare reaction. Late cutaneous reactions can occur 3 to 4 hours after challenge but these are not normally considered in routine diagnostic work.

In the skin prick test a drop of solution containing the allergen is placed on the volar aspect of the forearm. The skin is gently pricked with a needle (Pepys, 1975) or lancet (Osterballe and Weeke, 1979) through the solution without causing bleeding. The treatment site is scored 15 minutes and 25-30 minutes later by measuring the size of the weal (Dreborg, 1989). Weals greater than or equal to 3 mm are normally regarded as positive although any weal and flare reaction which is consistently larger than that produced by the solvent alone can be considered positive. A wide range of standardised skin prick test antigens are commercially available. However, if non-commercial skin test reagents are used it is important to select non-irritant concentrations of allergens by performing prick tests on non-allergic control subjects. The results of the skin prick test can be influenced by the skin prick test technique and antiallergic drug treatment.

Other less commonly used *in vivo* tests are the intradermal test, the rub test and the scratch test.

There are a number of *in vitro* tests for the measurement of specific IgE antibody in serum using radiolabelled or enzyme labelled anti-human IgE antibody. The *in vitro* tests are generally regarded

as less sensitive and more expensive than the *in vivo* tests. However, the results are semi-quantitative and objective, and are not influenced by the individual clinical condition or by medication. The most common assay is the radioallergosorbent test (RAST), a solid phase radioimmunoassay in which allergen is coupled to a solid phase such as a paper disk, polystyrene or insoluble cellulose (Newman *et al*, 1987). The solid phase allergen is incubated with patients serum and then with radiolabelled anti-IgE. The amount of radioactivity bound is proportional to the amount of specific IgE antibody in the patients serum. False negative results may be obtained because of competition with higher levels of other antibody isotypes (Grammer *et al*, 1989) and false positive results can be obtained by non-immune-specific binding of IgE (Karol, 1981b; Danks *et al*, 1981).

B.6 OTHER LABORATORY STUDIES

Additional studies should be considered in all patients and performed where appropriate. These may include complete blood count, chest x-ray, sputum examination and stain for eosinophilia, nasal secretion and stain for eosinophils, rhinoscopy and/or sinus x-ray.

B.7 OCCUPATIONAL ASTHMA: DIAGNOSTIC PROCEDURE

The subcommittee on "Occupational Allergy" of the European Academy of Allergology and Clinical Immunology suggested a diagnostic procedure that includes five steps (Maestrelli, 1992):

1. history suggestive of occupational asthma;

2. confirmation of bronchial asthma
   - reversibility of bronchial obstruction
   - non-specific bronchial challenges
   - serial measurements of peak expiratory flow rate;

3. confirmation of work-related bronchoconstriction
   - serial measurements of peak expiratory flow rate
   - serial measurements of non-specific bronchial reactivity;

4. confirmation of sensitisation to occupational agents
   - skin tests
   - *in vitro* tests (specific IgE or IgG);

5. confirmation of casual role of occupational agents
   - specific bronchial challenges.

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